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NEUROSECRETORY CELL TYPES AND DISTRIBUTION IN UNFED FEMALE HYALOMMA DROMEDARII (ACARI : IXODOIDEA : IXODIDAE) SYNGANGLION*+1

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+ Dedicated to late Dr. Harry Hoogstraal who initiated and supported this work before he passed away on 24 February 1986.

ABSTRACT

Neurosecretary Cell

Thirteen NSC types have been observed in the unfed female *Hyalomma dromedarii* synganglion. Distribution of these types was based on cell shape, size, and staining reaction of their contents. The NSC are grouped in 13 centers, namely protocerebral, cheliceral, stomodeal pons, palpal, oesophageal, globular, olfactory glomerular, four pedal, opisthosomal and postoesophageal. Each center contains one or more cell types.

INTRODUCTION

Gabé (1955) was the first to describe secretory activity within nerve cells in the synganglion of the argasid ticks *Ornithodoros (Pavlovskyella) erraticus* Lucas and *O. (Alveonasus) lahorensis* Neumann. Subsequently, neurosecretory cells (NSC) have been described in the ixodids *Boophilus (Uroboophilus) microplus* (Canestrini) (Binnington and Tatchell, 1973), *Dermacentor (D.) pictus* Herm (Ioffe, 1964), *D. (D.) variabilis* Say (Obenchain, 1974a, b; Obenchain and Oliver, 1975), *Hyalomma (H.) asiaticum* Schulze and Schlottke *Ixodes (I.) ricinus* (Linnaeus) (Ioffe, 1964), *I. (I.) persulcatus* Schulze (Panfilova, 1980), *Rhipicephalus (R.) sanguineus* (Latreille) (Chow and Wang, 1974), and the argasids *Argas (Persicargas) persicus* Oken (Eisen et al., 1973), *O. (O.) savignyi* (Audouin) (Evans and Solomon, 1977) and *O. (P.) parkeri* Cooley (Pound and Oliver, 1982).

The first attempt to classify the NSC into distinctive types was in *O. (O.) moubata* Murray (Eichenbergr, 1970). Later, Obenchain (1947b) presented an arbitrary classification scheme for the NSC in *D. variabilis* and Gabbay and Warburg (1977) in *O. (P.) tholozani*.

In *H. (H.) dromedarii* Koch, changes in the neurosecretory system have been correlated briefly with moulting and oviposition (Dhanda, 1967). In this study, we describe in detail the NSC types and their distribution in the synganglion of unfed female *H. dromedarii* to provide background data essential for subsequent studies on changes in these cells associated with feeding and mating.

MATERIAL AND METHODS

Adult *H. dromedarii* were colonized in the NAMRU-3 Medical Zoology laboratory from females collected from camels in the Imbaba market, Giza, Egypt, using domestic rabbits as hosts. Methods of feeding and rearing were those of Berger et al. (1971) at $28 \pm 1°C$ and 75% relative humidity. All animal care and manipulations were in accordance with the Animal Welfare Amendment of 1976 (PLP14-279) with subsequent amendments.

For histological examination, ticks were dissected on the day of moulting in a 0.7% saline solution and the synganglia were fixed in Bouin's fluid, dehydrated and double-embedded in celloidin-paraplast. Five to 7 μ-thick serial sections were stained using Mallory triple stain (MT) (Pantin, 1959), Ewen's (1962) paraldehyde-fuchsin (PF) and Gomori's (1941) chrome-haematoxylin-phloxine (CHP).

Cellular diameter of 6-10 specimens were measured. The surface area of sections of oblong cells was calculated using the formula $A = \pi \times \frac{a \times b}{4}$ where *A* is the area, *a* is the small diameter and *b* is the large diameter. The *mean and standard error were then calculated for each cell type* and the data were compared using the Student's *t*-test.

RESULTS

Thirteen NSC types (I-XIII) were observed in different locations in the synganglion. Distinction of these types was based on cell shape, size and staining reaction of their contents as illustrated in Table 1.

Type I : These NSC are the largest of all cell types ($P < 0.001$). The monoaxonal cell is oval, and contains several peripheral vacuoles and a large central nucleus with 3 nucleoli (Fig. 1). Some of the densely-distributed neurosecretory granules (NSG) clump together forming larger aggregates around the nucleus and others are traced in the axon.

Type II : These NSC are oval with a central nucleus having 2 nucleoli. Two subtypes are observed according to cell size and NSG amount and distribution. Subtype IIa cells have very fine granules enclosed in areas of clear, unstained cytoplasm which gives the cytoplasm a vacuolated appearance (Fig. 1). Smaller subtype IIb cells ($P < 0.02$) contain coarse granules with large accumulates on one side of the nucleus (Figs. 1, 4).

Type III : These NSC are large, oval and their nuclei contain 2 nucleoli. Two subtypes are distinguished according to cell size and NSG shape and distribution. In the larger subtype IIIa cells ($P < 0.01$) the cytoplasm contains several large vacuoles and the coarse NSG aggregate more densely at one side of the nucleus (Fig. 1). The subtype IIIb contains clumps of flocculant granules uniformly distributed in the cytoplasm (Figs. 1, 5).

Type IV : These are monoaxonal, more or less oval, each containing a large vacuole and an eccentric nucleus with 2 nucleoli (Figs. 1, 6). The fine NSG mainly aggregate near the cell periphery and in the axon.

Type V : These are large, irregularly-shaped cells, each containing 2 large vacuoles and a large central nucleus with one large nucleolus (Figs. 2, 7). The fine floculant NSG aggregate mostly at 2 sides of the nucleus.

Type VI : These are small, oval cells, with eccentric, spherical nuclei. Three subtypes are distinguished according to NSG distribution and/or cell size. In subtype VIa, the NSG are uniform and evenly distributed (Figs. 2, 8). In subtype VIb some of the fine NSG accumulate around the nucleus (Fig. 2). In subtype VIc large aggregates forming droplets are scattered among the fine granules (Fig. 2).

Type VII : These are the smallest ($P < 0.001$) NSC. They are rounded with relatively large nuclei and large NSG. Two subtypes VIIa and VIIb are observed according to NSG distribution ; the granules are scattered evenly in the cytoplasm of the former and irregularly in the latter subtype (Fig. 2).

Type VIII : These are monoaxonal cells in which 2 subtypes are observed according to NSG distribution ; the NSG are evenly scattered in subtype VIIIa (Figs. 2, 8) and accumulate in one side of the cell in subtype VIIIb (Fig. 2).

Type IX : In these oval cells 2 subtypes are distinguished according to cell size and NSG distribution. The NSG are enclosed in areas of clear, unstained cytoplasm in the smaller ($P < 0.01$) subtype IXa cells (Figs. 2, 9) and are distributed more or less evenly in the cytoplasm of the larger subtype IXb cells (Figs. 2, 10).

Type X : These are oval, unipolar cells with a homogeneous cytoplasm which contains a peripherally located vacuole and a central nucleus with 2 small nucleoli. The fine to coarse NSG are irregularly distributed in the cytoplasm (Fig. 2).

Type XI : These are oval cells with relatively large, rounded central nuclei, each containing 1 or 2 large nucleoli (Fig. 3). The cytoplasm contains several small peripherally-located vacuoles. The fine NSG are distributed throughout the cytoplasm with heavy aggregates at one side of the nucleus.

Type XII : These are irregularly-oval cells with oval eccentric nuclei. Each containing 3 large nucleoli (Fig. 3). The fine NSG are scattered throughout the cytoplasm with colloidal droplets among them.

Type XIII : Two subtypes are observed in these irregularly-outlined cells according to their size and number of axons. The relatively smaller ($P < 0.01$) polyaxonal subtype XIIIa cells and the large monoaxonal subtype XIIIb cells possess a central nuclei, each with 2 nucleoli (Figs. 3, 11). The cytoplasm contains several small vacuoles mainly at the cell periphery. The NSG are more heavily aggregated around the nucleus.

NSC distribution in the synganglion centers

Within the different cortical regions of the synganglion, the NSC are grouped in 13 centers. These groups are bilaterally symmetrical, each containing one or more cell types. Figure 12 and Table 2 illustrate the distribution of 22 neurosecretory groups in these centers.

Protocerebral center. This center includes 4 pairs of bilaterally symmetrical groups. *Group 1,* is the dorsal optic which consists of 2 or 3 type VIIa and 2 or 3 type X cells. *Group 2,* is anteroventral and consists of 4-6 NSC. It includes 2 type IIb cells, 1 or 2 type VIc cells and one type X cell. *Group 3,* is posterodorsal and consists of one type VIIb and 2 type XII cells. *Group 4,* is posterolateral and consists of 4 cells type IV, IX, X and XII and 2 type VIIb cells.

Cheliceral center. This center contains 3 pairs of bilaterally symmetrical groups. *Group 5,* is anterodorsal and consists of 2 type VIIa cells and one type IXa cell, *Group 6,* is ventral and consists of only one type IXa cell, while *Group 7,* is lateral and consists of 4 NSC of types VIa, VIIa, VIIIa and IXb.

Stomodeal pons center. Two pairs of bilaterally symmetrical groups occur in this center. *Group 8,* is dorsal and consists of 2 type VIIa cells, and *Group 9,* is ventromedial and consists of 3 type VIIa cells.

Palpal center. This center contains 2 symmetrical groups. *Group 10,* is dorsolateral and consists of one type IIIa cell, and 3 or 4 type VIIIb cells, and *Group 11,* is ventrolateral and consists of 2 type V cells and one type VIIa cell.

Oesophageal center. This center includes the bilaterally symmetrical *Group 12,* which lies ventrolateral to the oesophagus and consists of 1 or 2 type VIIa, 2 or 3 type VIIb, and 2 or 3 type XI cells.

Table 1 : Properties of the different neurosecretory cell (NSC) types in unfed female *Hyalomma dromedarii* synganglion.

Cell type	Mean cell dimensions (um) ± SE	NSG*	NSC Staining reactions PF**	CHP**	MT**
I	55.0±0.03x23.5±0.02	Very fine granules	Dark purple	Pale blue	Blue
IIa	30.8±0.02x22.0±0.03	Fine granules	Bluish purple	Blue	Reddish blue
IIb	27.5±0.02x20.9±0.03	Coarse granules	Bluish purple	Blue	Reddish blue
IIIa	44.0±0.03x30.0±0.04	Fine to coarse granules	Purple to dark purple	Pale blue	Blue
IIIb	35.0±0.03x25.0±0.03	Clumps of flocculent granules	Purple to dark purple	Pale blue	Blue
IV	29.0±0.03x15.5±0.02	Fine granules	Orange to purple	Blue	Pale blue
V	42.5±0.03x32.5±0.03	Fine flocculent granules	Orange purple	Blue	Pale blue
VIa,b	15.0±0.04x11.0±0.03	Fine granules	Purple to reddish orange	Blue	Reddish blue
VIc	18.0±0.03x15.0±0.04	Fine granules with large droplets among them	Purple to reddish orange	Blue	Reddish blue
VIIa	12.5±0.04x11.5±0.02	Large granules	Purple to dark purple	Blue to dark blue	Blue to dark blue
VIIb	11.5±0.02x10.0±0.02	Large granules	Purple to dark purple	Blue to dark blue	Blue to dark blue

(Cont. Table 1)

Cell type	Mean cell dimensions (um) ±SE	NSG*	NSC Staining reactions PF**	CHP**	MT**
VIIIa	20.0±0.02x 8.0±0.20	Coarse granules	Dark reddish purple	Blue	Pale blue
VIIIb	20.0±0.02x15.0±0.03	Coarse granules	Dark reddish purple	Blue	Pale blue
IXa	15.0±0.03x 7.5±0.20	Coarse granules	Purple to blue	Blue	Reddish blue
IXb	24.2±0.02x 8.8±0.20	Coarse granules	Purple to blue	Blue	Reddish blue
X	17.5±0.03x10.0±0.02	Fine to coarse granules	Orange purple	Pale blue	Reddish blue
XI	27.5±0.03x22.5±0.03	Fine granules with heavy aggregates	Dark purple	Pale blue	Blue to dark blue
XII	22.5±0.03x10.0±0.02	Fine granules with colloidal droplets among them	Purple or orange	Blue	Blue or red
XIIIa	33.0±0.03x26.5±0.03	Fine to medium granules	Bluish purple	Pale blue	Reddish blue
XIIIb	41.8±0.02x25.3±0.03	Fine to medium granules	Bluish purple	Pale blue	Reddish blue

*NSG = Neurosecretory granules

**PF = Ewen's paraldehyde-fuchsin, CHP = Gomori's chrome hematoxylin phloxine, MT = Mallory Triple stain.

Table 2 : Distribution of the neurosecretory cells (NSC) in the unfed female *Hyalomma dromedarii* synganglion*.

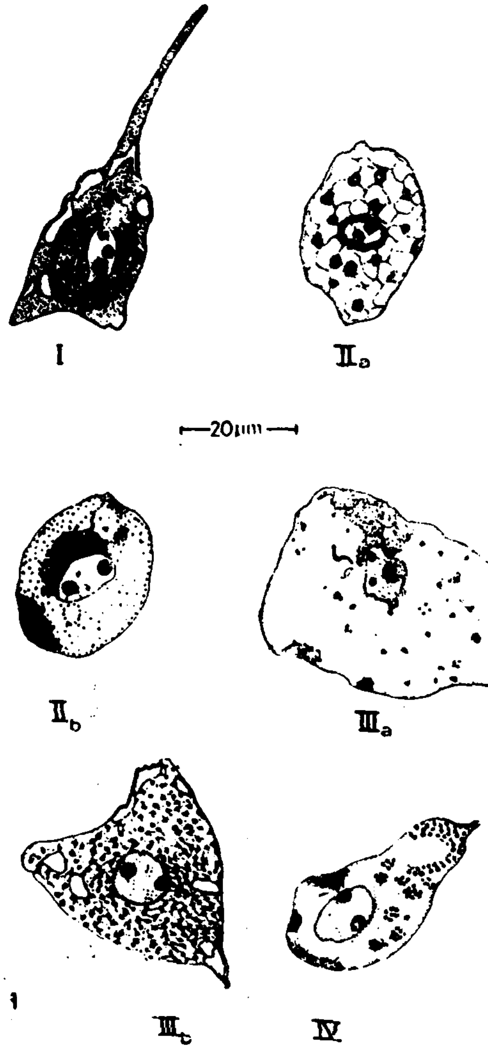
Center and group No.	Location		NSC No. in each group	NSC type and location
Protocerebral center				
Group 1	Dorsal optic	(paired)	4—6	2-3 VIIa mid-dorsal, 2-3 X dorsolateral
Group 2	Anteroventral	(paired)	4—6	2 IIb, 1-2 VIc, 1 X, anterodrosal to oesophages at its entry into synganglion
Group 3	Posterodorsal	(paired)	3	1 VIIb, 2 cells XII, dorsal to posterodorsal glomeruli
Group 4	Posterolateral	(paired)	6	1 IV, 2 VIIb, 1 IXa, 1 X, 1 XII, dorsolateral to anterodorsal glomeruli
Cheliceral center				
Group 5	Anterodorsal	(paired)	3	2 VIIa, 1 IXa, near neurilemma
Group 6	Ventral	(paired)	1	IXa, near posterior margin
Group 7	Lateral	(paired)	4	1 VIa, 1 VIIa, 1 VIIIa, 1 IXb, near neurilemma

(Cont. Table 2)

Center and group No.	Location		NSC No. in each group	NSC type and location
Stomodeal Pons center				
Group 8	Dorsal	(paired)	2	VIIa
Group 9	Ventromedial	(paired)	3	VIIa, surrounding oesophagus at its entry to synganglion
Palpal center				
Group 10	Dorsolateral	(paired)	4—5	1 IIIa, 3-4 VIIIb
Group 11	Ventrolateral	(paired)	3	2 V, 1 VIIa, near anterior margin of first pedal ganglia
Oesophageal center				
Group 12	Ventrolateral	(paired)	6—7	1-2 VIIIa, 2-3 VIIb, 2-3 XI, after oesophagus enters the synganglion
Globular center				
Group 13	Anteroventral	(paired)	2—3	1 VIIa, 1-2 VIIb
Olfactory glomerular center				
Group 14	Ventral	(paired)	6	2 IIb, 2 VIIb, 2 XIIIb

(Cont. Table 2)

Center and group No.	Location		NSC No. in each group	NSC Type and Location
Pedal center				
Group 15	Dorsolateral	(paired)	4	1 IIIa, 1 IIIb near neurilemma, 1 VIb deeper in cortex, (1 VIIa, 1 VIIb) adjacent to neuropile
Group 16	Ventrolateral	(paired)	4	2 VIIa adjacent to antero median surface of following ganglion, 2 VIIb near neurilemma
Opisthosomal center				
Group 17	Dorsomedial	(unpaired)	5—7	2 IIb, 1 IV, 3 VIIa,b, 1 VIIIb
Group 18	Dorsolateral	(paired)	2	1 IIIb, 1 XIIIa
Group 19	Ventromedial	(unpaired)	7—9	1-2 I, 5 VIIa, 1 XII, 1 XIIIb
Group 20	Ventrolateral	(paired)	6—7	3-4 VIIa, 3 VIIIb
Group 21	Posterior	(unpaired)	9	3 IIa, 2 VIIa, 2 VIIb, 1 XIIIa, 2 XIIIb
Postoesophageal center				
Group 22	Postoesophageal	(paired)	8—10	4 IIb, 1 IIIa, 3-5 VIIa, posterior to oesophagus exit from synganglion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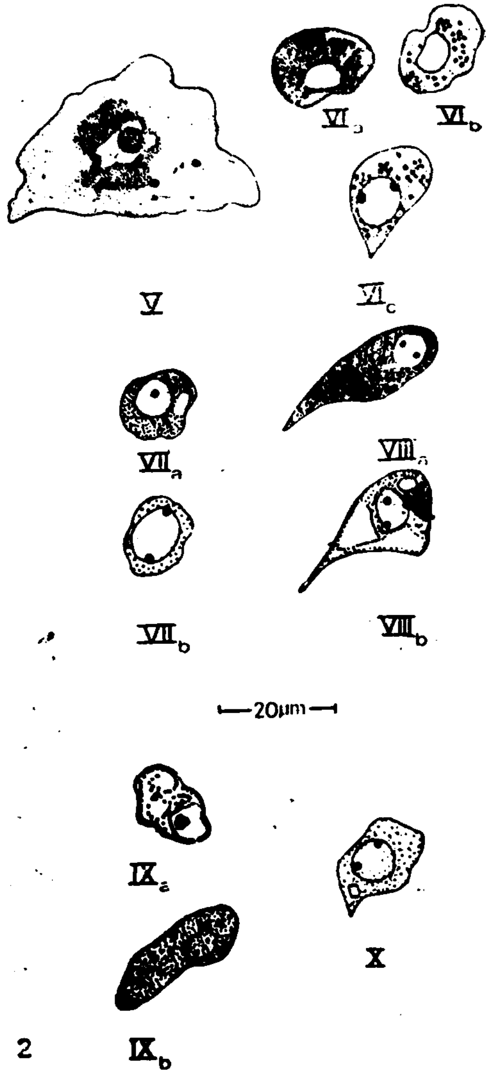
IIIc
IV


VI_a
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IX_a
X
IX_b

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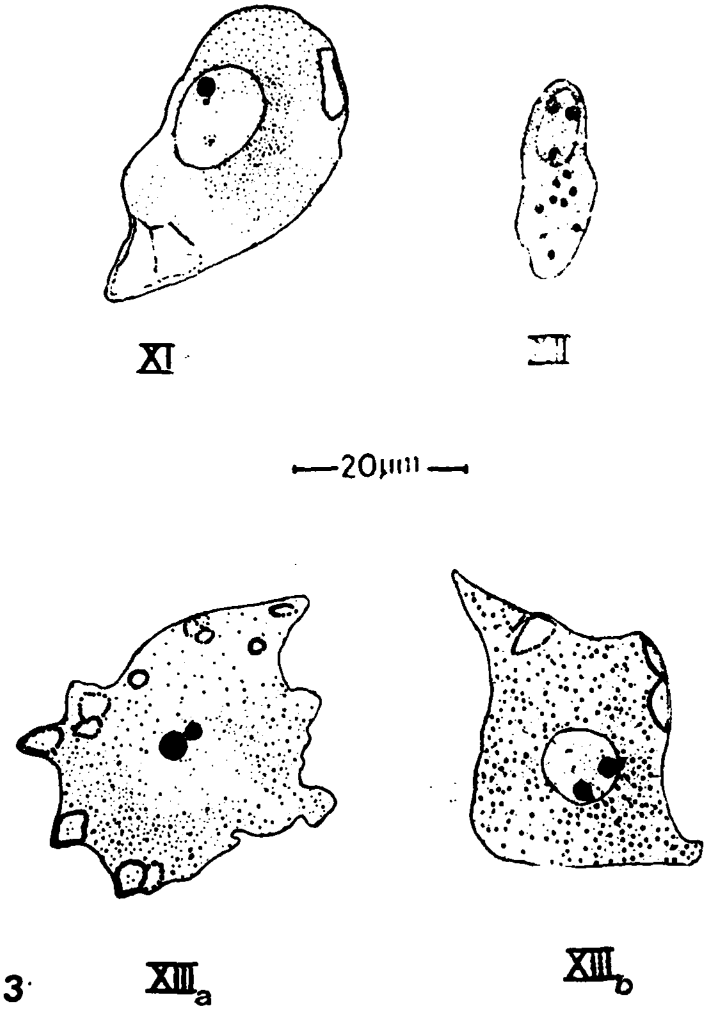

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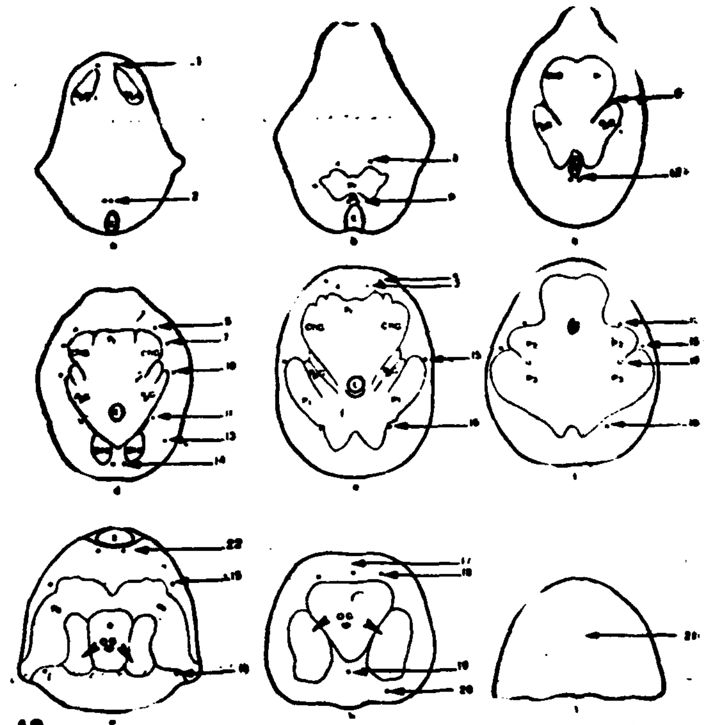

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FIGURES

Figs. 1-3 : Diagrammatic illustrations of the neurosecretory cell types I-XIII in unfed female *Hyalomma dromedarii.*

Figs. 4-11 : Transverse sections in the synganglion of unfed female *Hyalomma dromedarii* showing different neurosecretory cell types (arrows). X1500.
4. Type IIb. 5. Type IIIb. 6. Type IV. 7. Type V. 8. Types VIIIa (a) and VIa (b). 9. Type IXa. 10. Type IXb. 11. Type XIIIa.

Fig. 12 : Diagrammatic illustrations (a-i) showing the distribution of the neurosecretory groups (1-22) in the different ganglia of unfed female *Hyalomma dromedarii* synganglion. ChG, cheliceral ganglia ; E, oeophagus ; OG, opisthosomatic ganglion ; OfK, olfactory knots ; OpG, optic ganglia ; P 1-4, first to fourth pedal ganglia ; Pp G, pedipalpal ganglia ; protocerebrum ; St, stomodeal pons.

Globular center. This center contains one pair of bilaterally symmetrical *Group 13,* which is anteroventral to the globular cells and consists of 1 type VIIa and 1 or 2 type VIIb cells.

Olfactory glomerular center. This center contains one pair of bilaterally symmetrical *Group 14.* This is ventral and consists of 2 type IIb, 2 type VIIb, and 2 type XIIIb cells.

Pedal centers. In each of the 1st, 2nd, 3rd and 4th pedal ganglia occur 2 pairs of bilaterally symmetrical groups (15 and 16) with a similar pattern of NSC distribution. *Group 15,* lies dorsolateral in each pedal ganglion and consists of 5 cells, types IIIa, IIIb, VIb, VIIa and VIIb. *Group 16,* lies ventrolateral in each pedal ganglion and consists of 2 type VIIa cells and 2 type VIIb cells.

Opisthosomal center. This center contains 3 unpaired and 2 paired, bilaterally symmetrical groups. The unpaired *Group 17* is dorsommedial and consists of 5-7 NSC, 2 type IIb, one type IV, 1 type VIIa, 2 type VIIb, and one type VIIIb cells. The paired *Group 18,* is dorsolateral and consists of one type IIIb and one type XIIIa cell. *Group 19,* is unpaired and ventromedial; it consists of 7-9 cells, 1 or 2 type I, 5 type VIIa, one type XII cells and one type XIIIb. *Group 20* is paired and ventrolateral; it consists of 6-7 NSC ; 3 or 4 cells type VIIa and 3 type VIIIb. *Group 21,* is unpaired and posterior ; it consists of 3 type IIa, 2 type VIIa, 2 type VIIb, one type XIIIa and 2 type XIIIb cells.

Postoesophageal center. This center contains a pair of bilaterally symmetrical *Group 22,* which consists of 4 type IIb, one type IIIa and 3-5 type VIIa cells.

DISCUSSION

Thirteen different NSC types have been observed in the unfed female *H. dromedarii* synganglion according to the cell shape, size and staining reaction of their contents. Quantitatively, these types are similar to those described in the ixodid *Dermacentor variabilis* (Obenchain, 1974b) and the argasid

Ornithodoros tholozani (Gabbay and Warburg, 1977). Only 2 types, A and B, were found in *O. moubata* (Eichenberger, 1970) one type in *A. persicus* (Eisen et al., 1973) and 2 types α and β in *R. sanguineus* (Chow and Wang, 1974).

The form, quantity and/or distribution of the NSG in most of the different NSC types in *H. dromedarii* differ from those observed in other ixodid ticks (Obenchain, 1974b). Nevertheless, the NSG chromophilia in certain cell types in *H. dromedarii* is similar to that observed in certain NSC types of *D. variabilis* (Obenchain. 1974b) and *O. tholozani* (Gabbay and Warburg, 1977), yet most NSC in *H. dromedarii* are larger than those described in *D. variabilis*. Types I, II, VII and XI among *H. dromedarii* NSC contain NSG which stain purple to dark purple with PF, and are thus, similar to types I, VII, IX and X in *D. variabilis* (Obenchain, 1974b) and type II in *O. tholozani* (Gabbay and Warburg, 1977). This chromophilia indicates that the secretory material contains high levels of cysteine (Obenchain, 1974b).

Comparison between PF staining reactions of NSG in cell types of both *H. dromedarii* and *D. variabilis* (Obenchain, 1974b) indicates that types VI in the former stains purple to reddish orange as type V in the latter, while type VII in the former resembles types II and XI in the latter ; they all stain dark reddish purple. Types II, IX, and XIII in *H. dromedarii* stain bluish purple as types III, IV and VI in *D. variabilis* and types I and V in *O. tholozani* (Gabbay and Warburg, 1977). NSG staining reddish or bluish purple with PF seem to have a higher carbonyl content than those which stain dark purple (Obenchain, 1974b).

Types IV, V, X, XII in *H. dromedarii* contain an acidophilic secretory product with a staining affinity to the orange G in PF technique, thus simultating type XIIIa in *D. variabilis* and type X in *O. tholozani* (Obenchain, 1974b ; Gabbay and Warburg, 1977). Acidophilia persisting after oixdation may be attributed to proteins rich in tryptophan (Raabe and Monjo, 1970 ; Baudry and Baehr, 1970). Cytoplasmic affinity for the light green counterstain of CHP technique does not exist in *H. dromedarii*, although present in *D. variabilis* (Obenchain, 1974b) and *O. tholozani* (Gabbay and Warburg, 1977).

Grouping of NSC varies in ixodids, being fifteen groups in *B. microplus* (Binnington and Tatchell, 1973), eighteen groups in *D. pictus* (Ioffe, 1964), eighteen centers in *D. variabilis* (Obenchain and Oliver, 1975), and eighteen centers in *I. persulcatus* (Panfilova, 1981). Dhanda (1967) recognized eighteen NSC centers in *H. dromedarii* synganglion, without specifying their locations, but here we observed only thirteen centers containing 22 groups in this tick.

The fifteen and eighteen groups of NSC observed in *B. microplus* and *D. pictus*, respectively (Binnington and Tatchell, 1973 ; Ioffe, 1964) are recognizable within the thirteen NSC centers here described in *H. dromedarii* as well as the eighteen centers in *D. variabilis* (Obenchain and Oliver, 1975). Differences in the centers number in the two latter ixodids is mainly due to the inconsistent classification of NSC in the cheliceral, pedipalpal, and opisthosomal ganglia and stomodeal pons, into more than one center. In *D. variabilis* (Obenchain, 1974b) each neurosecretory component contains single typed-cells whereas in *H. dromedarii* it contains one or more cell types. As suggested by Obenchain (1974a) the small numbers of cells in each neurosecretory group seems to reflect an inherent economy which is also expressed in the condensation and miniaturization of acarine central nervous system.

In the present study, NSC presently demonstrated in the unfed female *H. dromedarii* require further study to elucidate their activity in hormonal secretions during and after feeding and their role in the regulation and coordination in the physiological events occurring during these phases. Studies on these parameters are underway.

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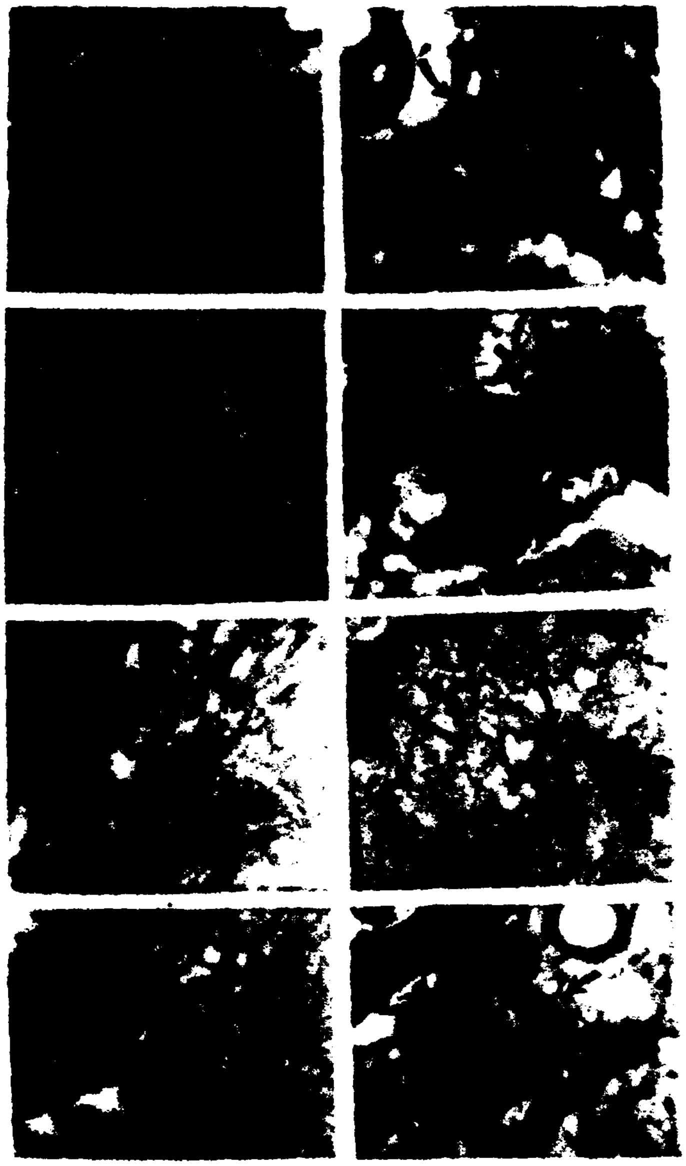

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Thirteen NSC types have been observed in the unfed female Hyalomma dromedarii synganglion. Distribution of these types was based on cell shape, size and staining reaction of their contents. The NSC are grouped in 13 centers, namely protocerebral, cheliceral, stomodeal pons, palpal, oesophageal, globular, olfactory glomerular, four pedal opisthosomal and post-oesophageal. Each center contains one or more cell types.

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